Characterization of Aster Yellows Phytoplasma Strains in Leafy Green Crops in Ohio

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Abstract

Aster yellows, caused by the aster yellows phytoplasma (AYP) is an important disease of lettuce and other vegetable crops in Ohio and other vegetable growing areas. It is transmitted primarily by the aster leafhopper, *Macrosteles quadrilineatus*. Seven strains of AYP (AY-WB, AY-S, AY-BW, AY-SS, AY-BD2, AY-BD3, and AY-SG) were identified previously in Ohio lettuce. The objective of this research was to determine the current diversity and distribution of AYP strains among leafy green vegetables in northwest Ohio. Two AYP strain-specific multiplex PCR assays (CPA1 and CPA2) were developed using primers previously designed to differentiate between two aster yellows (16SrI) subgroups. The CPA1 assay distinguishes AY-WB and AY-S while the CPA2 distinguishes AY-BD2 and AY-WB. In addition, both assays detect a strain or strains not yet characterized that may be novel. The multiplex PCR assays were used to characterize 584 infected leafhoppers collected from two different sites in northwest Ohio. AY-BD2 infected leafhoppers were the most abundant (55%) followed by AY-WB (36%). Only one leafhopper infected with AY-S was detected and AYP strains infecting the remaining leafhoppers (9%) were not identified using these assays. AY-BD2 infected leafhoppers were predominant in red lettuce, whereas AY-WB infected leafhoppers were predominant in romaine lettuce. There was an interaction between host, AY-strain and site (p= 0.00).

Introduction

Research Goal:

To understand the diversity and the distribution of AYP strains in leafy green crops in Ohio

Objectives:

To develop an AYP strain-specific multiplex PCR assay.

Determine relative abundance and diversity of AYP strains in leafhoppers collected in leafy vegetables over the growing season.

To determine if the host crop has an effect on AYP strain distribution.

Hypotheses:

The proportion of different AYP strains will vary in leafhoppers collected from leafy green crops in Ohio.

Relative AYP distribution will change during the growing season.

AYP strain distribution will vary by location.

AYP strain distribution will be influenced by host.

Material and Methods

Insect collection

Insects were collected from romaine, red leaf, and green leaf lettuce and cilantro and parsley grown in Hartville and Celeryville on a bi-weekly schedule during Summer 2008.

DNA extraction

Genomic DNA from field-collected insects, cage-reared insects infected with reference strains , healthy leafhoppers and healthy aster tissue was extracted using the Wizard SV96 extraction kit. Extracted DNA from five leafhopper samples was pooled for PCR.

AY - specific nested PCR amplification

Pooled samples were amplified using the AYP-specific-primers P1/P7 **(1,4)** followed by R16F2n/R2 **(2,3)**. Individual samples from each positive-nested PCR were then tested individually using AYP-specific and nested PCR as described above.

Multiplex PCR development

Two multiplex PCR assays **(CAP1, and CAP2; Figure 1)** were developed using previously published AYP strain specific primers **(5).** PCR products were separated by horizontal gel electrophoresis .

Statistical Analysis

One way ANOVA was used to evaluate the interaction between the sampling time, host, AYP strains and location using SAS software.

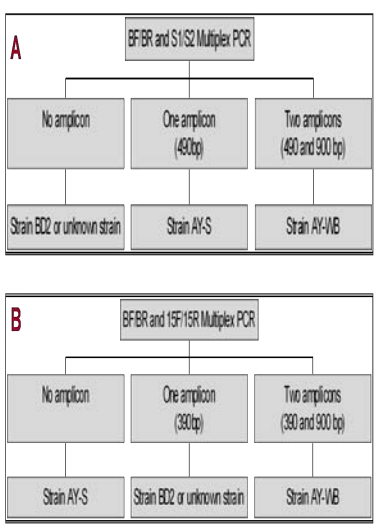

Figure 1: Multiplex PCR scheme to distinguish strains of AYP in leafy greens. Scheme A. (CAP1) used to identify AY-S and AY-WB. Scheme B. (CAP2) used to identify AY- BD2 and AY-WB

Results

Table 1. Percentage of AYP Infected Leafhoppers Collected from Fields in Ohio in 2008

AYP strains	Percent infected
AY-BD2	55
AY-WB	36
AY-S	0.1
AYP unidentified strains	9
Total	58.4

AYP Strain Abundance

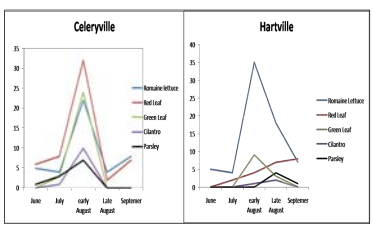

Figure 2: Abundance of AYP positive leafhoppers in leafy greens during the growing season, Ohio 2008.

•In Celeryville: AYP- infected leafhoppers were most abundant in early August in red leaf lettuce (p=0.00).

•In Hartville: AYP- infected leafhoppers were most abundant in late August in all leafy green (p=0.00) except for romaine and green leaf lettuce, which was in early August.

AYP Strain Diversity

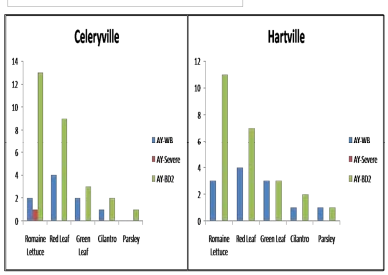

Figure 3: AYP strains in leafhoppers collected from leafy greens.

•In both locations, AY-BD2 predominant in leafhoppers in all leafy greens (p = 0.00).

AYP Strain Distribution

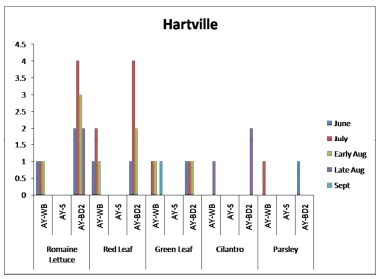

Figure 4: Distribution of AYP Strains in Leafy Greens Over Time in Hartville.

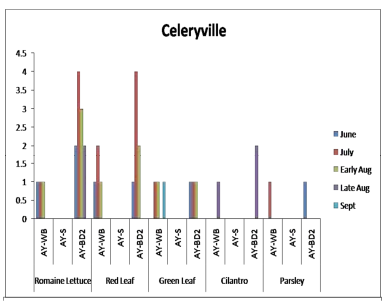

Figure 5: Distribution of AYP Strains in Leafy Greens Over Time in Celeryville.

In both locations:

There were significantly higher numbers of leafhoppers infected with AY-BD2 than other strains in Cilantro.

In red leaf lettuce , AY-BD2- infected leafhoppers were significantly more abundant in July than in other months.

Conclusions

In Celeryville and Hartville:

The host and the sampling time influenced the abundance and distribution of AYP strains over the growing season.

The proportion of different AYP strains varied in leafhoppers collected from leafy green crops in Ohio.

The distribution of AYP strains changed over the growing season , but there were no significant differences between locations .

Reference

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